**INTRODUCTION**

Testicular cancer (TC) is a rare tumor among men, representing only 1-2% of all tumors. The National Cancer Institute (NCI) reported 8,850 new cases in 2017 and this number is estimated to increase to 9,310 new cases in 2018. TC can be classified into germ cell tumors, non–germ cell tumors, and extragonadal tumors. Non-Seminomatous Germ Cell Tumor (NSGCT) or Testicular Cancer Non-Seminoma (TCNS) is an aggressive testicular germ cell tumor. On the other hand, Testicular Germ Cell Tumour Seminoma (TCSS) or Testicular Cancer Seminoma (TCS) is the most common type of testicular cancer that grows and spreads slowly. Sperm banking is recommended in TC patients prior to cancer treatment. There is no literature describing the proteins related to the reproductive functions in the sperm of TCNS patients. We, therefore, evaluated the sperm proteome and their role in altered semen quality in both TCNS and TCS patients undergoing sperm banking, before starting cancer treatment, by high throughput proteomics and functional bioinformatics approaches.

**MATERIALS and METHODS**

After getting institutional approval, semen samples from men with TCS (n=15) and TCNS (n=15) were obtained before sperm banking for this proteomic analysis using Finnigan LTQ-Orbitrap Elite Hybrid Mass Spectrometry. After semen analysis, samples were prepared for proteomic analysis. Spermatozoa were isolated and proteins were extracted, lysed and quantified using LC-MS/MS (Figure 1). Western blot (WB) was performed using individual samples from the TCSS and TCS groups (n=6/group). Bioinformatic analysis of DEPs identified by LC-MS/MS was carried out using the Ingenuity Pathway Analysis (IPA) Software.

**RESULTS**

1. Semen parameters in TCS and TCNS group are shown in Table 1.
2. High throughput proteomic analysis results are shown in (Figure 2).
3. Chaperonins of the T-complex protein-1 (TCP-1) family (CCT2, CCT3, CCT4, CCT5, CCT6A, CCT6B, CCT7, CCT8) were upregulated in TCS compared to TCNS group. These subunits mediate capacitation-dependent binding of spermatogenesis to the zona pellucida and, thus, essential for fertilization (Figure 3). Hence, we can predict that the expression of TCP-1 family of proteins in both TCS and TCS could be reduced when compared to the normal control group.
4. Based on the IPA analyses, we selected six proteins based on the abundance and expression pattern by the proteomic analysis (Table 2).
5. WB results showed underexpression of UQCRC2 (P=0.039), HSPA2 (P=0.032) and SPA17 (P=0.018), as well as the overexpression of MMP9 (P=0.039) in TCS group (Figure 4 and Table 3).

**CONCLUSIONS**

1. Overexpression of MMP9 correlates with the higher invasiveness observed in TCS relative to TCNS.
2. Decreased expression levels of UQCRC2, HSPA2, and SPA17 may explain why the fertility problems are usually more severe in patients with TCNS.
3. The altered expression levels of these proteins are associated with spermatogenesis dysfunction, reduced sperm-kinetics and motility, failure in hyperactivation, capacitation, and fertilization.
4. These proteins may serve as a new diagnostic tool to distinguish between TCS and TCNS.

**SUPPORT**

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